Appendix 3

Pharmatox

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IN VITRO SKIN IRRITATION

of Emulsion B8575

Submitted to:
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1. SUMMARY

The SKINTEX method is an *in vitro* test used to predict skin irritation, based on both the alterations of a protein matrix and release of a dye. The potential of Emulsion B8575 for skin irritation was investigated in the Skintex Upright Membrane Assay (UMA).

The sample was found to be a minimal irritant.

2. INTRODUCTION

2.1. Sponsor

EGO Pharmaceuticals Pty. Ltd.

2.2. Project number

Project **T1716.1.B.**

2.3. Sample Description

Emulsion B8575, a white cream stored at room temperature (between 19 and 24° C).
The reactivity and physical data were determined by EGO Pharmaceuticals Pty. Ltd.

2.4. Rationale of the study

To determine the potential for dermal irritation caused by the test substance in the SKINTEX™ System.

2.5. Basic principle

The SKINTEX barrier matrix of collagen and keratin is highly organized. An indicator dye molecule which is similar to the stratum corneum is linked into this matrix. Chemical irritants can interact with the SKINTEX reagent. The dye release can be quantitated spectrophotometrically. The SKINTEX Reagent is a protein reagent which undergoes a process of conformational change when challenged with a chemical irritant. The endpoint of this process is the opacification of the reagent, which can also be measured spectrophotometrically. The sum of the dye release and opacification of the reagent is determined on a colorimeter. Calibrators provide a direct comparison to a Draize Primary Dermal Irritation Index (PDII) Scale to determine Dermal Safety Classifications.

2.6. Protocol selection and procedural summary

The samples are analysed by direct application to the barrier matrix and incubation of the matrix in contact with the reagent. Several modifications are possible with this protocol which permit the most accurate analysis of the sample.

Calibrators and Controls

Three calibrators and two Quality Control Samples are analysed in each assay to ensure standardization.

Qualification

The protocol has qualification steps which must be completed and fulfilled for a result to be accepted. Classification and determination of the SKINTEX/Draize Equivalent Primary Dermal Irritation Index(PDII) is then carried out.

3. RESULTS

The SKINTEX/Draize PDII for Emulsion B8575 was **0.3.** The dose response curve for the sample can be found in the Appendix.

4. CONCLUSION

The SKINTEX Upright Membrane Assay was carried out on a sample of Emulsion B8575.
The sample was found to be a minimal irritant.

Appendix

- Dose Response Curve
- Equivalence Table

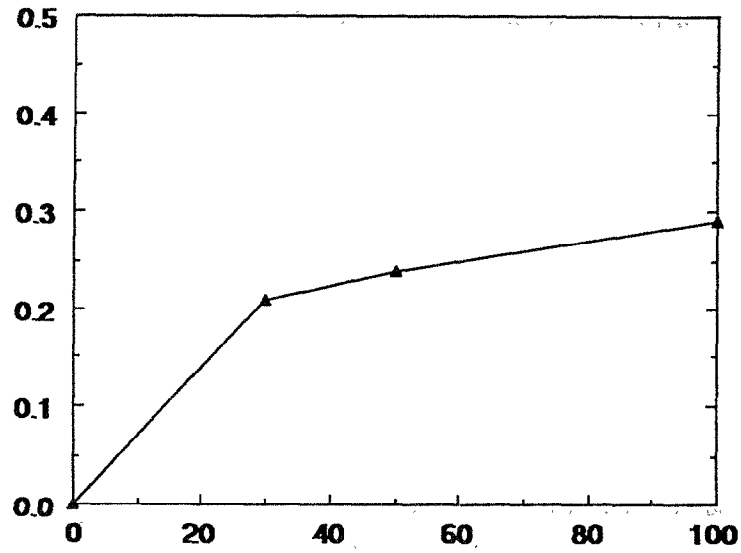
Sample: Emulsion B8575
Company: EGO Pharmaceuticals Pty. Ltd
Code: T1716.B
Protocol: UMA/Skintex
Date: 09/03 /1995

Sample result: Volume: 100 µl Classification: Minimal
PDII EQUIVALENT: 0.29

Cuvette	Sample OD	Blank OD	Net OD	Score	Classification
SKO	72				
SK1	85				
SK2	308				
SK3	1076				
100 µl	34	-16	50	0.29	Min
50 µl	28	-12	40	0.24	Min
30µl	22	-14	36	0.21	Min

Senior Technician: F.Brook

Dose response curve



SKINTEX/DRAIZE EQUIVALENCE

The Primary Dermal Irritation Index (PDII)/Equivalent is based on the Draize scale.

Minimal	0 - 0.5
Mild	0.5 - 2.0
Moderate	2.0 - 5.0
Severe	≥ 5

All test samples with *in vitro* Draize equivalents which are ≥ 0.5 are positive results or potential irritants.